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SCIENCE CAREER

FluRox based biosensors

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Think of a biosensor and youwould conjure up with the question of sensitivity. Nusrat Sanghamitra, Fellow of EdRox network explains how a simple but yet novel concept of detecting redox reaction induced fluorescence change shoots up the sensitivity.

Abstract A biosensor is an integrated device of biomaterials and electronic components which detects physiological change or physico-chemical response. The efforts towards the development of a supersensitive FluoRox biosensor are discussed in this paper. FluoRox principle is based on the novel concept of monitoring redox events in vitro and in vivo by fluorescence detection based on forster resonance energy transfer (FRET). Unlike conventional electrochemical biosensors fluorescence based sensors has the advantage of higher sensitivity which under suitable conditions can detect single molecules. Thus a highly sensitive and a miniaturized device is aimed at, which will enable the detection of trace amounts of pollutants and the detection of diseases at an early stage.

Keywords Fluorescence · Biosensors · Redox protein · P450 · Hemocyanin · Nitric oxide

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1 Ultrasensitive biosensors for…

"Prevention is better than cure" is an age old saying but it rightly acknowledges the challenge that the world is facing today: the rapidly growing need of sensitivity in the detection devices or biosensors deployed in various areas like diagnosis of diseases and environmental monitoring of industrial pollutants etc. A biosensor is a device that detects, records, and transmits information regarding a physiological change or the presence of various chemical or biological materials in the environment. More technically, a biosensor is a probe that integrates a biological component with a physico-chemical component to detect an analyte.

The Marie Curie Research Training Network, EdRox aims at the development of an ultrasensitive biosensor by exploiting the FluoRox principle. Unlike typical electrochemical sensors (the well known Glucose biosensor (Boutelle et al. 1996), these FluoRox biosensors implement the novel concept of monitoring redox events in vitro and in vivo by fluorescence detection based on forster resonance energy transfer (FRET). A leap in sensitivity can be achieved by fluorescence detection, which has reached the point of observation of single molecules under suitable conditions. Thus, it not only has the promise of increasing sensitivity by orders of magnitude, but it also allows miniaturization to the sub-micrometer level, thereby opening up new areas of technological applications.

2 Working principle

Essentially all life processes are fuelled by redox reactions which is also important in the non-living world. The operating principle of FluoRox biosensors is based on optical detection i.e., fluorescence intensity (ON/OFF) of a fluorescently labeled redox protein or enzyme immobilized on an electrode following a redox event.

sensitivity and efficiency of optical detectors. Increasing the sensitivity by one to three orders of magnitude will enable the detection of trace amounts of pollutants and the detection of diseases at an early stage. It will have an impact on environmental issues and medicare.

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In the FluoRox principle, the redox protein is labeled with a fluorescent dye (Fig. 1). Excitation light hits the label and the absorbed energy is emitted in the form of fluorescence. The fluorescence intensity is quenched or not, depending on the redox state of the active center (M). A change in redox state of the active center (M) leads to a change in fluorescence. The redox state changes when the active center exchanges electrons either with the electrode or with the analyte. Thus, the change can be translated into the fluorescent intensity of the label covalently attached to the protein surface on the basis of FRET (Kuznetsova et al. 2006). Hence, the label lights up (ON) or dims (OFF) if a redox event takes place: the fluorescence signals the passage of electrons on their way from the electron donor to the active site of the enzyme or vice versa (Fig. 1). Therefore, the enzyme activity can be monitored by following fluorescence; instead of electrons we monitor photons, i.e., detection is transferred from the electrochemical to the optical domain. We can therefore utilize the superior

3 Application fields

This method can be potentially applied to any prosthetic group in a redox protein that changes in absorption spectrum upon oxidation/reduction by judicious selection of a fluorescent label and a carefully chosen label attachment point. Thus, this principle was exploited towards development of an oxygen biosensor for high-throughput monitoring of cell growth and antibacterial drug screening (Strianese et al. 2009). This oxygen biosensor is a copper protein known as hemocyanin which has a binuclear copper center that binds a molecule of oxygen. Upon binding to oxygen, the absorption of hemocyanin changes and this change in absorption can be translated into a change in fluorescence intensity of an attached label through the FRET mechanism. In the deoxygenated protein, all the energy absorbed by the label is emitted as fluorescence i.e., the label is 'ON' or lights up whereas upon oxygenation, a part of the absorbed energy gets transferred to the metal

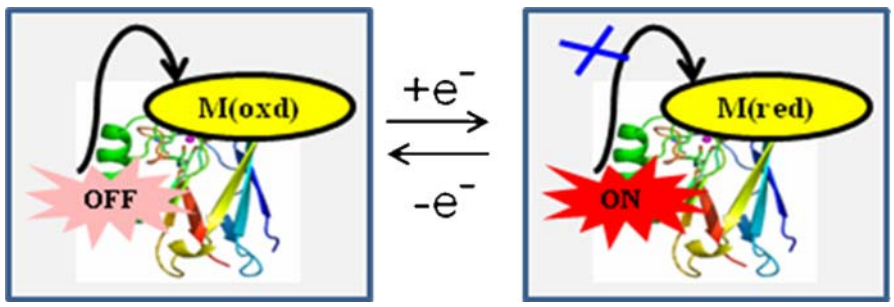

Fig. 1 Schematic representation of the FluoRox principle

center thereby resulting in a diminished fluorescence intensity and the label becomes dim and turned 'OFF'. Thus in an aerobic bacterial culture, the biological oxygen consumption by bacteria, in other words the cell growth and viability is sensed by the fluorescent intensity of a labeled hemocyanin. It also serves as a faster way to perform antibiotic screening for drug discovery which could have possible clinical applications (Fig. 2).

The FluoRox based method was also explored to detect the substrate binding of cytochrome P450, a heme protein monooxygenase, which is an important class of enzyme owing to its diverse reactivity. It catalyzes a wide variety of physiologically important processes such as fatty acid metabolism, xenobiotic degradation and steroid biosynthesis etc. It is also industrially important since it uses a plethora of both exogenous and endogenous compounds as substrates in enzymatic reactions. The proof of principle has been provided by the detection of substrate bound and unbound form of cytochrome P450cam which binds to the substrate camphor. Since substrate binding is the first step of the catalytic activity it is important to accurately determine the binding affinity of a potential substrate to the enzyme in order to assess its potential for catalytic conversion.

Similarly the reduction and oxidation of a copper containing enzyme nitrite reductase corresponding to the conversion of nitrite to nitric oxide was also studied which can be developed as a potential nitric oxide (NO) sensor (Kuznetsova et al. 2008). Most

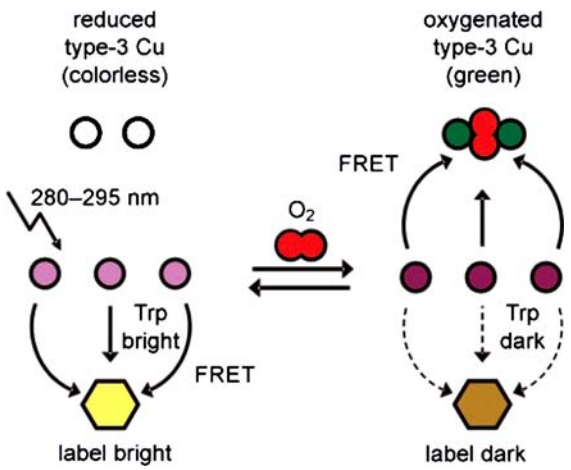

Fig. 2 Schematic representation of FRET based oxygen sensing (Zauner et al. 2007)

importantly, the FluoRox principle was also successfully applied towards the single molecule mechanistic study, where enzymatic turnover of surface immobilized single copper containing nitrite reductase was studied for the first time (Kuznetsova et al. 2008).

4 Towards an engineered bio-nanoelectrode

Translation of the novel FluoRox concept to a platform technology requires a wide range of expertise in the fields of molecular biology, biochemistry, biophysics, nanofabrication and materials science. The concomitant research efforts of the whole consortium is a cooperative effort towards production of proteins/enzymes suitable for FluoRox purposes, construction of electrodes with tailor-made surfaces, immobilization of proteins on the electrodes and characterization of the engineered electrodes. The topography, integrity and functionality of the electrodes are characterized by various state of the art techniques like advanced scanning probe techniques, atomic force microscopy (AFM), scanning tunneling microscopy (STM), electrochemical analysis and fluorescence microscopy.

5 Training of young researchers

The Research Training Network, EdRox not only aims at achieving a super sensitive prototype FluoRox biosensor for a wide range of applications, but is also committed to provide state-of-the-art training in bio-nanotechnology to early stage researchers (ESR) and career crossover skills to experienced researchers (ER). Training includes teaching of complementary skills like communication and presentation, research management, handling of intellectual property (IP), teaching, entrepreneurship, and exploration of career opportunities. Educational programs and goals, including personal career development plans, are individually tailored for each ESR and ER fellow in the network. Besides, visits of fellows to the laboratories of partner members of the consortium is encouraged and facilitated. Thus, the whole research and training program of EdRox is designed in such a way that it not only gives an opportunity to the fellows to get trained and gain expertise in a wide range of techniques but also provides them with a

Table 1 Partners of EdRox

Partner	Website	Expertise	Country
Prof. Dr. G. W. Canters Leiden Institute of Chemistry, Leiden University	http://protchem…s.net/index.php	Protein Engineering, mechanism of redox enzymes	The Netherlands
Prof. Dr. T. A. Aartsma Leiden Institute of Physics, Leiden University	http://www.biophysics.leidenuniv.nl/aartsma-bf/	Fluorescence based biosensors and photosynthesis	The Netherlands
Prof. Dr. G. Gilardi, Department of Biology, University of Torino	http://www.unito.it/dba/giagilar.htm	Engineering novel redox protein for bionanotechnology	Italy
Dr. J. J. Davis, Department of Chemistry, Oxford University	http://www.chem.ox.ac.uk/researchguide/jjdavis.html	Molecular electronics, nano-scale chemistry and sensory interfaces	United Kingdom
Prof. Dr. Marco Sola, Department of Chemistry, University of Modena	http://155.185.2.170/sitiwebgruppi/Sola/paginawebaprile2005/paginaweb0405.html	Bioinorganic chemistry, characterization of electron transport proteins	Italy
Dr. C. Dennison, Biomedical Sciences, Medical School, Newcastle University	http://www.ncl.ac.uk/camb/staff/profile/christopher.dennison	Structure and function of copper proteins, biological NMR	United Kingdom
Prof. Dr. I. Willner, Institute of Chemistry, Hebrew University of Jerusalem	http://chem.ch.huji.ac.il/willner/	Bioelectronics, DNA based biosensors, molecular switches and motors	Israel
Dr. L. C. Jeuken, Institute of Membrane and Systems Biology, University of Leeds	http://www.mnp.leeds.ac.uk/ljcjeuken/index.htm	Catalytic mechanism of membrane proteins	United Kingdom
Prof. Dr. H. A. O. Hill, Oxford Biosensors Ltd	http://www.oxford-biosensors.com/	New type of portable diagnostic devices for primary healthcare	United Kingdom
Prof. Dr. G. Robillard, Biomade Technology Foundation	http://biomade.nl/	Molecular nanotechnology	The Netherlands

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broader outlook and prospective of research as a career, which significantly adds to further career development.

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6 EdRox: the Marie Curie research training network consortium

EdRox stands for research and training in redox enzymology. The consortium (Table 1) consists of ten partners from eight universities throughout Europe including two SMEs (small to medium sized enterprises).

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